CervidTB Stat Pak® Implementation

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Safeguarding Animal Health

Why a New Serologic Test?

- ✓ Employ new accurate diagnostic test technology
- ✓ Minimizes capture and handling events for animal safety
- ✓ Expected to promote additional cervid TB testing
  - Requested by USAHA and cervid industry
- ✓ Comparable sensitivity and specificity to current skin tests

Historical Timeline

- Stat-Pak licensed for elk and red deer, 2009
  - White-tailed and fallow deer, 2010-11
- 2010 - USAHA resolution - USDA evaluate Stat-Pak as official TB test
- 2011 – Project to evaluate TB serologic tests in cervids (Cervid Serology Project); USAHA resolution to approve

Historical Timeline

- Oct 2012 – USDA licenses the Dual-Path Platform (DPP) secondary test for elk, red deer, white-tailed deer, and fallow deer
  - ➢ Improved specificity compared to Stat-Pak
- Oct 2012 – USDA approves the Stat-Pak (primary) and DPP (secondary) as official bovine TB tests in elk, red deer, white-tailed deer, fallow deer and reindeer

Cervid Serology Project Objective

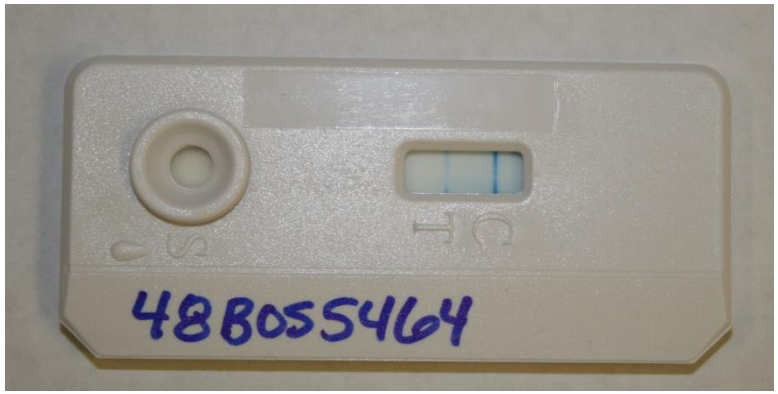
- Evaluate TB detection tests *for official bovine tuberculosis (TB) program use* in captive and free-ranging cervids
  - North American elk *(Cervus canadensis)*
  - White-tailed deer *(Odocoileus virginianus)*
  - Reindeer (*Rangifer tarandus*)
- Primary/screening test:
  - CervidTB Stat-Pak® (Stat-Pak)
- Secondary Test:
  - Dual Path Platform (DPP)

Background for Test

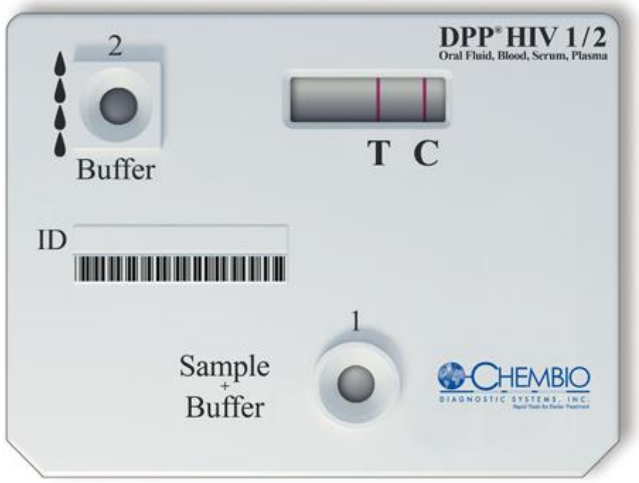
- Chembio Diagnostic Systems, Inc.

- Rapid serologic tests
  - Immunochromatographic, lateral-flow technology
  - Detect antibodies to *M. tuberculosis* and *M. bovis* proteins
  - Test performed on serum

Chembio Test Cassettes

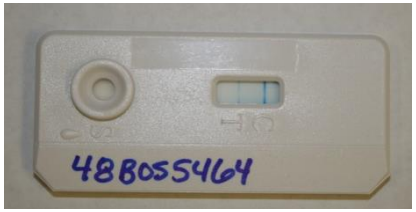


Stat-Pak



Dual-Path Platform (DPP)

Need for a Secondary Test



Stat-Pak as a 1°Test

CCT is not an appropriate secondary test.

- Cell-mediated versus humoral responses
- TB Committee SAS agrees

Necropsy as a confirmatory test

Licensure vs. Official Program Approval

- Licensure
  - Responsibility of the Center for Veterinary Biologics (CVB)
  - Tests are evaluated for sensitivity, specificity, ruggedness, repeatability, and suitability
  - Includes review of manufacturing process and test labels
  - Details are in VS Memo 800.73
- Official TB program approval
  - Focuses on sensitivity, specificity, and comparison to currently approved program tests
  - Collaborative effort with CVB, Regions, NVSL, CEAH, ARS, States
  - VS Memo 552.40

Cervid Serology Project

- 1,752 animals met the case definition for presumably uninfected
  - 873 elk, 725 white-tailed deer, and 185 reindeer
  - From 51 premises in 17 States
- 31 animals met the case definition for confirmed infected
- 52 TB-positive by Stat-Pak
  - 14 elk, 24 white-tailed deer, and 14 reindeer
  - 26 from banked samples
    - 5 elk, 11 white-tailed deer, and 10 reindeer
  - 26 prospectively sampled
    - 9 elk, 13 white-tailed deer, and 4 reindeer

Cervid Serology Project

- Evaluated ChemBio CervidTB Stat-Pak® during 2011
  - Licensed for elk, red deer, fallow deer, white-tailed deer, and reindeer
- 1,783 cervids tested
- Specificity estimates not significantly different than SCT*
  - 98.3%, elk,
  - 96.7%, white-tailed deer
  - 92.4%, reindeer
- Sensitivity estimates
  - 75-87%, elk
  - 55-67%, white-tailed deer
  - Not available for reindeer

*single cervical TB skin test

Presumably Uninfected, Necropsy Results

- 26 prospectively sampled animals, Stat-Pak positive
  - 9 elk
    - 7 necropsied. Histology microgranuloma (1), lymphoid hyperplasia (2), no significant findings (4).
  - 13 white-tailed deer
    - 4 necropsied. No significant findings on histology.
  - 4 reindeer
    - 1 necropsied. Histology microgranuloma.
  - Culture – *M. intracellulare* (2), unidentified atypical mycobacterium (elk origin samples)
- **NO** *M. bovis*-infected animals found

Remember - Sensitivity vs. Specificity?

- Sensitivity:
  - How likely a test will correctly identify truly diseased animals

(In low-TB prevalence populations like cervids, a negative result suggests with a high probability that the animal is truly negative)

Remember - Sensitivity vs. Specificity?

- Specificity:
  - How likely a test will correctly identify truly non-diseased animals

Presumably Uninfected

- 26 prospectively sampled animals
- Stat-Pak positive
- Culture negative for *M. bovis*

  - False positives do occur on Stat-Pak testing
  - Follow up with secondary DPP test
  - Combining the tests increases specificity

Dual Path Platform Results for 52 Stat-Pak Antibody Positive Samples

	Neg	Pos	Total	Series Specificity** (%)	Stat-Pak Specificity
Elk	14	0	14	842/842 (100)	98.34%
WTD***	19	5	24	720/725 (99.3)	96.69%
Reindeer	11	3	14	182/185 (98.4)	92.43%
Total	44	8	52	1744/1752 (99.4)	97.03%

**Combination of Stat-Pak and DPP results used in series
***White-tailed deer

SCT/CCT* and Stat-Pak/DPP Series Specificity

	SCT/CCT (95% CI)	Stat-Pak/DPP (95% CI)
Elk**	90.4% (87.4 – 92.9)	100% (98.3 – 100.0)
Deer**	87.1% (84.5-89.4)	99.3% (96.7 – 99.3)
Reindeer	Not available	98.4% (95.3 – 99.7)

*Reference by Norden, et al (1996)

**The Stat-Pak/DPP results are significantly different from the SCT/CCT results, $p < 0.05$.

Conclusions

- Stat-Pak specificity not significantly different than the SCT for elk, white-tailed deer, and reindeer
  - Specificity highest in elk > white-tailed deer > reindeer
- In this study the Stat-Pak sensitivity is significantly higher than the SCT in elk Specificity of Stat-Pak/DPP in series significantly higher than SCT/CCT in elk and deer
- Different animals are antibody-positive by Stat-Pak than are SCT responders

Citation

- Jeffrey T. Nelson, Kathleen A. Orloski, Audra L. Lloyd, Mark Camacho, Mark A. Schoenbaum, Suelee Robbe-Austerman, Bruce V. Thomsen, and S. Mark Hall: *Evaluation of Serodiagnostic Assays for Mycobacterium bovis Infection in Elk, White-Tailed Deer, and Reindeer in the United States,* Veterinary Medicine International Volume 2012 (2012)

- Link: http://www.hindawi.com/journals/vmi/2012/563293/

Sample Collection

Sample Collection (Cont'd)

- Who may collect and submit serum samples for CervidTB Stat-Pak® testing?
  - Only TB Designated Accredited Veterinarians (DAV)
  - Non TB DAVs, contact your VS Area Office to receive training to acquire DAV status
  - Current TB DAVs don't need additional training

Sample Collection (Cont'd)

- Specimen to be tested is serum
- Collect blood in 10 ml Red Top tube or serum separator tube

- Collect a minimum of 10 ml blood
- An additional tube of blood will be needed if submitting to lab for brucellosis testing

Sample Processing and Handling

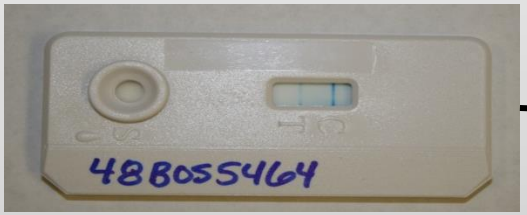
- Allow blood to clot at room temperature
- Centrifuge sample and remove serum from clot; place serum in a new clean tube
- Refrigerate serum samples; **Do Not Freeze**
- Label tube with sample number and official animal ID
- Keep samples refrigerated until shipped

Sample Processing and Handling (Cont'd)

- Refrigerated serum should be shipped to NVSL within 24 to 48 hours
- Do not ship to arrive at NVSL over the weekend
- Excessive hemolysis in the serum may make samples untestable

Samples Testing Positive on Initial Stat-Pak Test

- Serum samples testing positive on Stat-Pak will be tested with the secondary test



- The DPP secondary test will be performed on the same serum sample
- → No need to capture and restrain the animal a second time

Sample Submission to NVSL

- Initially NVSL will be the only testing lab
- Submission form is a VS Form 10-4/10-4a
- Record tube/sample number and official ID for each animal on the form
- Inventory lists, spreadsheets, or VS Form 6-22 (TB test record) may be attached to the VS 10-4/10-4a in lieu of listing each animal on the form

Sample Submission to NVSL (cont'd)

- VS Form 10-4/10-4a can be downloaded at http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml
- TB Stat-Pak specimen submission kits are available from NVSL for approximately $30 by:
  - Calling 515-337-6200
  - Emailing NVSL_userfee@aphis.usda.gov

Sample Submission to NVSL (cont'd)

- Cost of submission/shipping kit also includes the cost of return shipping to NVSL
- Ship specimens by next-day or 2nd-day delivery
- Ship UPS or FedEx

Sample Submission Information

- Contact your VS Area Office for:
  - Permission to send samples to NVSL
  - Instructions and forms
    - VS Form 10-4
    - VS Form 10-4a
    - VS Form 6-22 (TB test record)
  - Information for enrolling herds in the TB Qualified or Accredited Herd program
  - Designated Accredited Veterinarian training

State Regulations

- The use of official tests in States is conditional upon the approval of the State Animal Health Official
- Confirm with the State Veterinarian if the Stat-Pak and DPP tests are currently accepted in that state

Test Results

- Test results will be provided to the testing veterinarian within 4 to 5 business days by email, fax, or mail. Copies will also be sent to State animal health officials, State AVICs, and Area epidemiologists.
- Veterinarians may specify preferred method to pay for tests and get results on the submission form or when they set up their accounts with NVSL by calling 515-337-6200.

FAQs

- Q. Can the Stat-Pak and DPP be used on any species of cervid?

- A. The Stat-Pak and the DPP secondary test are approved for use in elk, red deer, white-tailed deer, fallow deer, and reindeer.

FAQs (cont'd)

- Q. Is the Stat-Pak test approved for mule deer or sika deer?

- A. No. The Stat-Pak and the DPP secondary test are approved for use only in elk, red deer, white-tailed deer, fallow deer, and reindeer.

FAQs (cont'd)

- Q. What is the advantage of using the Stat-Pak test over the single cervical skin test?

- A. Deer, elk, or reindeer only have to be handled one time so a blood sample can be collected versus two handling events for the skin tests – one for tuberculin injection and a second for interpreting the tests.

FAQs (cont'd)

- Q. If I am also testing the animals for brucellosis, may I just collect one blood sample and submit it to NVSL?

- A. No. When also testing for brucellosis, an additional tube of blood must be collected and submitted to an approved brucellosis testing laboratory accompanied by a properly completed VS Form 4-33.

FAQs (cont'd)

- Q. What happens if the Stat-Pak test is positive?

- A. If the Stat-Pak has a positive result, NVSL will perform the secondary DPP serological test using the serum from the same sample that was originally submitted. If the DPP is positive, an epidemiological investigation will be done. The disposition of the animal will be determined by the Area and Regional TB epidemiologists according to TB program regulations.

FAQs (cont'd)

- Q. Can the Stat-Pak test be run on cervids that respond to the single cervical skin test?

- A. No. Cervids classified as responders (positive) on the single cervical skin test must be retested using the comparative cervical skin test. Likewise, cervids positive on the Stat-Pak test must be retested using the DPP secondary test.

FAQs (cont'd)

- Q. If an animal was recently skin tested for TB, can I perform the Stat-Pak test?

- A. No. You must wait a minimum of 90 days after the animal was skin tested before you can retest with either the Stat-Pak test or another skin test.

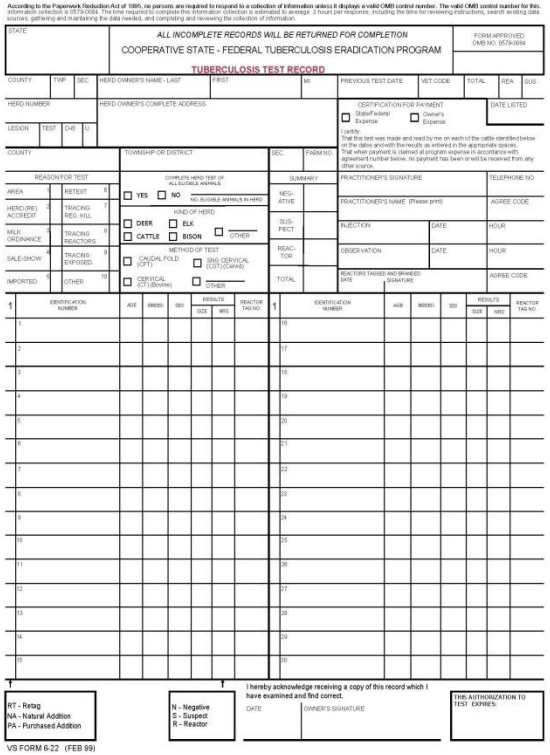
Additional Actions

- 9 CFR 77.20 is being amended to approve the Stat-Pak and DPP as official TB program tests.
- A guidance document to provide instructions for using the tests has been furnished to the State Veterinarians and AVIC's and is available on the USDA – APHIS TB website.

Forms

VS 10-4

VS10-4a
(Continuation Sheet)



VS 6-22
(TB test record)

When Will Serologic Testing For Cervids be Available?

- CVB previously licensed the CervidTB Stat-Pak®
- DPP test was licensed on October 12, 2012
- Sample collection and submission to NVSL will begin on XX-XX-2012.

Questions and Comments